Regional transcapillary albumin exchange in rodent endotoxaemia: effects of fluid resuscitation and inhibition of nitric oxide synthase

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ABSTRACT

Sepsis is characterized by increased microvascular permeability and regional variations in capillary perfusion, which may be modulated by nitric oxide (NO) and reversed by fluid resuscitation (FR). The effects of saline FR and NO synthase blockade [by $\text{N}^\text{G}$-nitro-$\text{L}$-arginine methyl ester (L-NAME)] on microvascular albumin transport and perfused capillary density were assessed in anaesthetized Wistar rats with acute normodynamic endotoxaemia. Separate dual-isotope techniques were employed to measure the permeability index (PIA) and the permeability × surface area product index (PIB), which provide different and complementary information regarding blood–tissue albumin exchange. PIA represents the tissue/blood distribution volume ratio of albumin. PIB is a composite measure of endothelial permeability and the vascular surface area available for albumin exchange, and therefore takes into account the effect of altered blood volume. Capillary density was quantified by fluorescence microscopy following circulation of Evans Blue-labelled albumin. Compared with controls, PIA was reduced significantly in lipopolysaccharide (LPS)-treated animals in skeletal muscle and skin, probably due to blood volume redistribution rather than to changes in permeability. PIB was increased significantly in LPS-treated animals in the kidney, mesentery, skeletal muscle, skin and lung, and in the small bowel following FR. FR also improved the LPS-induced metabolic base deficit, but did not alter capillary density. L-NAME significantly attenuated the LPS-induced rise in PIB in the lung. In conclusion, acute endotoxaemia induces tissue-dependent variations in microvascular albumin exchange. FR improves acid–base disturbance in endotoxaemia, through mechanisms other than microvascular recruitment. NO appears to increase microvascular permeability in endotoxaemia, an effect that may be attenuated by L-NAME, particularly in the lung.

INTRODUCTION

Sepsis is characterized by increased microcirculatory permeability and regional variations in capillary perfusion [1]. Clinically, the development of tissue oedema may be seen in association with organ dysfunction. The development of multiple organ system failure frequently follows, to which most patients succumb [2]. Patients with sepsis also develop endothelial cell damage and dysfunction. This leads to down-regulation of constitutive enzyme systems, such as constitutive nitric oxide synthase (cNOS). Nitric oxide (NO) modulates vascular...
tone under physiological conditions, but induction of inductive forms of NOS (iNOS) in vascular smooth muscle and overproduction of NO is thought to induce refractory hypotension and maldistribution of microcirculatory blood flow. NO may also modulate capillary integrity and therefore cause tissue oedema formation, leading to impaired oxygen utilization [3]. Currently, patients with sepsis are supported by fluid resuscitation (FR) to preserve tissue perfusion, and are frequently treated with pressor agents to maintain vascular tone [4].

Using rodent models, we and others have shown that endotoxaemia produces vascular abnormalities similar to those found in the clinical state [5]. However, changes in microvascular permeability to protein are poorly characterized and may vary between different capillary beds [6]. Some variability may arise from the nature of the quantitative techniques employed to date, and from the manner in which permeability changes are calculated and therefore defined. Moreover, in studies carried out under physiological conditions, investigators have distinguished between blood-borne and tissue-distributed tracer molecules, but in sepsis there is evidence of blood redistribution and a fall in plasma volume, with increased spatial and temporal heterogeneity of microcirculatory flow [7,8]. Sepsis also potentially alters the volume of distribution of markers of permeability by influencing tissue hydration and lymphatic drainage.

The aims of the present study were therefore threefold. The first was to obviate the methodological difficulties outlined above by employing complementary tracer-labelled methods of measuring endotoxin-induced changes in transcapillary albumin transport; quantifying albumin flux and changes in capillary permeability and perfused capillary density (CD) respectively. Secondly, we wished to assess the effect of FR on the measured indices. Finally, we examined the possible role of NO in modulating microvascular permeability in the same model.

METHODS

Animals
Male Wistar rats (Charles Rivers Ltd, Hythe, Kent, U.K.) weighing 200–300 g were housed in a temperature- (21 °C) and humidity-controlled environment. Food (Special Diet Services Ltd, Chelmsford, Essex, U.K.) and water were supplied ad libitum. The study protocols were approved by the Home Office (U.K.) licensing authority, and the care and handling of animals was in accordance with Home Office (U.K.) guidelines.

Drugs, gases and reagents
All laboratory gases were supplied by the British Oxygen Company (BOC, Sunningdale, U.K.) and certified pure. Lipopolysaccharide, (LPS; Salmonella enteritidis; code no. L6011), N⁶-nitro-L-arginine methyl ester (l-NAME), BSA (fraction V; Sigma) for conjugation with radioiodine and trichloroacetic acid were purchased from Sigma (Poole, Dorset, U.K.). Pentobarbitone sodium (Sagatal®) was obtained from Rhone-Merieux (Harlow, Essex, U.K.).

Preparation of radioactive tracers
Radioiodine-labelled BSA (1-albumin) was prepared by standard methods [9], and dialysed exhaustively to remove free radioactive iodine. The preparation was dialysed again immediately before use. Levels of unbound free radioiodine in the circulation, determined by trichloroacetic acid precipitation of terminal blood samples, were < 2%. Each animal was injected with 0.25 ml of sterile 0.9% NaCl containing ~10⁶ c.p.m. of ¹³¹I- albumin and the same volume containing ~10⁶ c.p.m. of ¹³¹I-albumin.

Surgical procedures and monitoring
Animals received either 20 mg/kg Salmonella enteritidis LPS or an equal volume (0.75 ml) of 0.9% NaCl (intraperitoneal) 240 min before the end of the experiment. They were anaesthetized with sodium pentobarbitone (60 mg/kg, intraperitoneal), and maintained at a core temperature of 36–38 °C on a thermostatically controlled heating pad. The left carotid artery and right jugular vein were cannulated using 0.75 mm external diam. polyethylene catheters (Portex, Hythe, Kent, U.K.). For continuous monitoring of mean arterial pressure (MAP), administration of radiolabelled tracers and blood sampling, the carotid cannula was attached to a pressure transducer (Hewlett Packard) via a three-way tap. A tracheostomy was performed and the rats were ventilated with 21% O₂/5% CO₂/74% N₂ (BOC), using a volume-cycled small-animal ventilator (Harvard Instruments, Chatham, Kent, U.K.). Following cannulation and institution of mechanical ventilation, a 15 min period of equilibration was allowed. Arterial blood (0.2 ml) was withdrawn periodically, and replaced by an equal volume of saline, to monitor arterial blood gases using a Radiometer ABL3 system blood gas analyser (Radiometer AC, Copenhagen, Denmark). In some instances, a further 0.2 ml was sampled for haematological and biochemical analyses. FR was achieved, where required, by the slow administration, over 1 min, of a 4 ml/kg bolus of 0.9% NaCl via the jugular vein at 120 min after administration of LPS (or saline), followed by infusion at a rate of 16 ml h⁻¹ kg⁻¹ [5]. In some experiments, 0.5 ml of l-NAME (1 mg/kg) was administered as an intravenous bolus just before the introduction of radioiodinated albumin at 180 min. This dose was shown previously to modulate microvascular changes (e.g. tissue oxygenation, tissue partial pressure of
O_2) induced by LPS in the same model [3,5]. Experimental procedures were terminated by an intravascular bolus of sodium pentobarbitone.

**Haematological and biochemical measurements**

Blood (0.20 ml) was taken at the end of Protocol A (see below) for measurement of haemoglobin, total white blood cell and platelet counts, haematocrit and serum lactate.

**Tissue water content**

The wet weight (W) of tissues was determined using an electronic balance (Mettler AT261 Delta range) after gently blotting dry. Samples were dried at 75 °C for 48 h, by which time no further reduction in weight was apparent, and then weighed to determine the dry tissue weight (D). The hydration index (HI) was determined as HI = (W – D)/D.

**Albumin permeability measurements**

Protocol A was designed to quantify tissue uptake of albumin, relative to intravascular uptake. Briefly, rats were divided randomly into six experimental groups, which were studied in random order: Group 1, sham-treated (n = 5; CON); Group 2, LPS-treated (n = 5; LPS); Group 3, sham-treated plus FR (n = 5, FR CON); Group 4, LPS-treated plus FR (n = 5; FR LPS); Group 5, sham-treated plus L-NAME FR plus FR (n = 5, L-NAME FR CON); Group 6, LPS-treated plus L-NAME plus FR (n = 5; L-NAME FR LPS). At 180 min after administration of saline or LPS, 0.25 ml of ^131^I-albumin was injected via the carotid cannula as a marker of permeability. At 235 min, the same volume of ^125^I-albumin was injected as an intravascular reference marker. At 240 min, an arterial blood sample was taken for analysis of arterial blood gases. Blood samples (1 ml) were also drawn for measurement of blood radioactivity, and of haematological and biochemical parameters. Terminal anaesthesia was administered and mechanical ventilation discontinued. Tissue samples from heart, lung, liver, kidney, spleen, mesentery, descending aorta, skeletal muscle and skin were harvested and cleared of surface blood contamination by briefly rinsing with saline and blotting dry. ^125^I and ^131^I radioactivity in blood and tissue samples was measured in a γ-radiation scintillation counter (Model S100; Canberra Packard, Pangbourne, Berks., U.K.), and standard corrections for crossover between counting channels and background were applied.

The permeability index (PI_A) was then calculated as:

$$PI_A = U_T^{125}/U_T^{131}$$

(1)

where tissue uptake of iodinated albumin (U_T) was calculated as U_T = N/C_P; N is the amount of I-albumin in the tissue (expressed as c.p.m./g dry weight) and C_P is the concentration of I-albumin in the plasma (expressed as c.p.m./ml of blood sample) at 240 min.

Protocol B was designed to provide an index (PI_B) of the permeability × surface area product (i.e. a composite measure of permeability and vascular area available for albumin exchange) of each microvascular bed, and represented a modification of a previously established method [2]. Briefly, Wistar rats were divided randomly into the same six experimental groups as in Protocol A (total n = 32). The experimental protocol was identical with that employed in Protocol A until 240 min after LPS/saline administration. At that point, the entire circulation was immediately washed through with heparinized saline (1000 units/l; pressure 100 cmH_2O), followed by 10% formaldehyde, through a purpose-built small stainless steel cannula passed via a left ventriculectomy to the aortic arch. Inspection of histological sections from all tissues removed confirmed that the microcirculation was cleared of blood by this procedure. The same tissues were harvested and assayed as in Protocol A, as well as sections of diaphragm, proximal jejunum, distal ileum and ascending colon. PI_B was calculated as:

$$PI_B = U_T^{131}/U_T^{125}$$

(2)

**Perfused CD**

Perfused CD was determined in skeletal muscle (right rectus femoris) from certain animals studied using Protocol B: Group 1 (n = 18 muscle sections from four animals; CON); Group 2 (n = 15 from four; LPS); Group 3 (n = 25 from five; FR CON); Group 4 (n = 26 from six; FR LPS). At 235 min, 0.25 ml of Evans Blue-labelled BSA (50 mg/ml), prepared by standard methods [10], was administered with the ^131^I-albumin. Following _in situ_ fixation, skeletal muscle blocks were sectioned and examined by fluorescence microscopy (Leitz Aristoplan, Wetzlar, Germany) at × 250 magnification. Digitized colour images of three or more sections from each muscle block were examined, and perfused capillaries were recognized as points of high fluorescence intensity. CD for each section was determined by point counting and referenced to muscle fibre area (Matlab®; Mathworks Inc., Natick, MA, U.S.A.).

**Statistical analysis**

Data are presented as means ± S.E.M. Multiple-group comparisons were performed using one-way ANOVA followed by the Student–Newman–Keuls post-hoc test to isolate differences. Differences between individual group means were tested by an unpaired Student’s _t_-test. _P_ values of < 0.05 signified significant differences. CD values are expressed as medians for group comparisons. The heterogeneity of capillary densities was defined as the range of values for which the coefficient of variation was calculated (mean ± S.E.M.).
RESULTS

Systemic MAP

Values for MAP, combined from animals enrolled in both Protocols A and B, are shown in Figure 1. L-NAME changed MAP significantly, causing a 20% rise from baseline at 180 min in L-NAME FR CON animals (Group 5; \( P < 0.01 \)), which was sustained until 240 min. This was also significantly higher than that in the CON animals at the 180 min time point (Group 5 compared with Group 1; \( P < 0.01 \)). MAP also rose 25% from baseline in L-NAME FR LPS animals (Group 6; \( P < 0.01 \)), and was significantly higher than that in LPS animals at 180 min (Group 6 compared with Group 2; \( P < 0.01 \)).

Table 1  Arterial blood gas analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Standard base excess (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CON</td>
<td>-4.21 ± 1.40</td>
</tr>
<tr>
<td>2</td>
<td>LPS</td>
<td>-7.77 ± 2.14</td>
</tr>
<tr>
<td>3</td>
<td>FR CON</td>
<td>-4.17 ± 2.01</td>
</tr>
<tr>
<td>4</td>
<td>FR LPS</td>
<td>-6.54 ± 1.13</td>
</tr>
<tr>
<td>5</td>
<td>L-NAME FR CON</td>
<td>-7.90 ± 1.68</td>
</tr>
<tr>
<td>6</td>
<td>L-NAME FR LPS</td>
<td>-10.42 ± 2.0</td>
</tr>
</tbody>
</table>

Arterial blood gas analysis

The results of arterial blood gas analysis, combined from animals enrolled in both Protocols A and B, are shown in Table 1. There were no statistically significant differences between groups. However, some interesting trends were apparent. The standard base excess of LPS-treated animals showed a trend to be lower than that of their corresponding controls (Groups 2, 4 and 6 compared with Groups 1, 3 and 5 respectively). Following FR, the standard base excess in LPS-treated animals showed a trend to improvement, from -7.77 to -6.54 mmol/l (Group 2 compared with Group 4). L-NAME plus FR attenuated this improvement, from -4.17 to -7.90 mmol/l (Group 3 compared with Group 5) and from -6.54 to -10.42 mmol/l (Group 4 compared with Group 6).

Haematological and biochemical results

The total white cell counts of all LPS-treated groups were significantly lower than those in the matched control groups at 240 min (Groups 2 and 4 compared with Groups 1 and 3 respectively; \( P < 0.01 \)) (Table 2). Similarly, platelet counts were lower in LPS-treated animals than in controls; significantly so after FR at 240 min (Group 4 compared with Group 3; \( P < 0.05 \)). Serum lactate in FR LPS animals was significantly higher than in controls (Group 4 compared with Group 1; \( P < 0.05 \)).

Tissue water content

The HI varied in a tissue-dependent fashion [range 3.7
Haematological and biochemical values were taken 240 min after sham (CON) or endotoxin (LPS) treatment alone, with FR or with \(\text{L}-\text{NAME} \) plus FR. Values are means \(\pm\) S.E.M. (all groups \(n = 5\)). Significance of differences: lactate: \(* P < 0.05\) for FR LPS compared with CON; white cell count: \(** P < 0.01\) for LPS, FR LPS and \(\text{L}-\text{NAME} \) FR LPS compared with CON; platelet count: \(*** P < 0.001\) for LPS, FR LPS and \(\text{L}-\text{NAME} \) FR LPS compared with CON. These values are also significantly different from those for the respective control groups (by ANOVA).

### Table 2  Haematological and biochemical values

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Haemoglobin (g/dl)</th>
<th>Haematocrit (l/l)</th>
<th>White cell count ((10^9/l))</th>
<th>Platelet count ((10^9/l))</th>
<th>Serum lactate (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CON</td>
<td>14.2 (\pm) 0.3</td>
<td>40.6 (\pm) 1.0</td>
<td>5.6 (\pm) 0.9</td>
<td>778 (\pm) 37</td>
<td>3.0 (\pm) 0.6</td>
</tr>
<tr>
<td>2</td>
<td>LPS</td>
<td>12.3 (\pm) 1.0</td>
<td>35.9 (\pm) 4.1</td>
<td>1.1 (\pm) 0.2**</td>
<td>277 (\pm) 25**</td>
<td>4.7 (\pm) 0.7</td>
</tr>
<tr>
<td>3</td>
<td>FR CON</td>
<td>14.1 (\pm) 0.7</td>
<td>40.5 (\pm) 1.5</td>
<td>6.7 (\pm) 1.8</td>
<td>651 (\pm) 32</td>
<td>6.8 (\pm) 1.6</td>
</tr>
<tr>
<td>4</td>
<td>FR LPS</td>
<td>13.4 (\pm) 0.3</td>
<td>37.8 (\pm) 1.2</td>
<td>1.9 (\pm) 0.3**</td>
<td>341 (\pm) 87**</td>
<td>7.5 (\pm) 1.7**</td>
</tr>
<tr>
<td>5</td>
<td>(\text{L}-\text{NAME}) FR CON</td>
<td>13.2 (\pm) 0.6</td>
<td>40.2 (\pm) 3.1</td>
<td>4.7 (\pm) 0.7</td>
<td>589 (\pm) 78</td>
<td>1.6 (\pm) 0.7</td>
</tr>
<tr>
<td>6</td>
<td>(\text{L}-\text{NAME}) FR LPS</td>
<td>12.7 (\pm) 0.7</td>
<td>37.9 (\pm) 2.3</td>
<td>1.3 (\pm) 0.2**</td>
<td>244 (\pm) 61**</td>
<td>5.0 (\pm) 1.0</td>
</tr>
</tbody>
</table>

### Figure 2  Permeability index to albumin (PI\(_A\))

Animals were randomly divided into sham-treated (CON) and endotoxin-treated (LPS) groups, with the following treatments: no FR, with FR or with \(\text{L}-\text{NAME} \) plus FR (\(\text{L}-\text{NAME} \) FR). Data are presented as means \(\pm\) S.E.M. (\(n = 5\) in all groups). MES, mesentery; SKEL, skeletal muscle. Significance of differences: \(* P < 0.05\) compared with CON.

(lung) to 1.5 (aorta)). However, there were no significant differences in HI between groups for any tissue.

### Albumin permeability indices (PI\(_A\) and PI\(_B\))

Protocol A experimental results are summarized in Figure 2. Group 1 (CON) and Group 2 (LPS) had a PI\(_A\) \(> 1\) in all tissues studied, implying a net flux of albumin into the tissue at 240 min. LPS reduced PI\(_A\) significantly in skeletal muscle and skin compared with controls (Group 2 compared with Group 1; \(P < 0.05\)). After FR, skeletal muscle and skin from LPS-treated animals again displayed a significant fall in PI\(_A\) compared with their respective controls (Group 4 compared with Group 3; \(P < 0.05\)). Following \(\text{L}-\text{NAME} \) administration, PI\(_A\) in skeletal muscle and skin from LPS-treated animals fell significantly compared with their controls (Group 6 compared with Group 5; \(P < 0.05\)), but had no effect in other tissues.

Protocol B experimental results are summarized in Figure 3. LPS-treated animals displayed an increase in PI\(_A\) in lung, kidney (medulla and cortex), mesentery, skeletal muscle and skin compared with controls (Group
Figure 3 Index of permeability × surface area product (PI

Animals were randomly divided into sham-treated (CON) and endotoxin-treated (LPS) groups, with the following treatments: no FR, with FR or with L-NAME plus FR (L-NAME FR). Kmed, renal medulla; Kcort, renal cortex; MES, mesentery; DIAPH, diaphragm; SKEL, skeletal muscle. Data are presented as means ± S.E.M. (n = 32 total). Significance of differences: *P < 0.05, †P < 0.01 compared with CON; ‡P < 0.05 compared with L-NAME FR CON.

Figure 4 Perfused CD in skeletal muscle

Animals were randomly divided into sham-treated (CON) and endotoxin-treated (LPS) groups, with the following treatments: no FR, with FR or with L-NAME plus FR (L-NAME FR). Data points represent individual skeletal muscle sections. The median value is presented for each group, in addition to the range (representing the heterogeneity of CD) (n = 5 in all groups). Significance of differences: *P < 0.05 compared with CON.

Following LPS, CD was significantly higher than in control animals (Group 2 compared with Group 1; P < 0.05) (Figure 4). Moreover, there was greater heterogeneity (range and coefficient of variation) in CD values in LPS compared with CON animals. After FR, the heterogeneity of CD in FR CON animals was increased compared with that in baseline CON animals (Group 3 compared with Group 1). However, FR had no additional effect in FR LPS animals (Group 4 compared with Group 2).

DISCUSSION

In this rat model, LPS produced vascular and metabolic changes consistent with clinical sepsis. Moreover, a significant, tissue-dependent increase in the permeability × surface-area product index (PI

2 compared with Group 1; P < 0.05) at 240 min. After FR, the PI

of aortae from FR LPS animals was higher than that in the same tissue from FR CON animals (Group 4 compared with Group 2; P < 0.01). In the lung, following L-NAME administration, the LPS-induced rise in PI

was completely reversed (Group 6 compared with Group 5; P < 0.05). There was a trend to a higher lung PI

in L-NAME FR CON animals than in L-NAME FR LPS (compare Groups 5 and 6).
transport ($\text{PI}_A$) was detected. Secondly, in LPS-treated animals, FR increased the range of tissues displaying a rise in $\text{PI}_B$, although not apparently via an effect on microvascular recruitment as represented by perfused CD. Finally, 1-NAME reduced LPS-induced albumin uptake ($\text{PI}_A$) within the lung, but may have worsened $\text{PI}_B$ in tissues from control animals.

This study employed two dual-isotope tracer techniques. $\text{PI}_A$ may be affected by alterations in tissue hydration, blood pressure or lymphatic exchange. However, with evidence of blood redistribution in sepsis [7] leading to the exclusion of albumin from certain vessels, $\text{PI}_A$ may also be influenced by endotoxaemia-induced changes in perfusion in highly vascular tissues. Furthermore, a fall in colloid oncotic pressure during sepsis may reduce the net transfer of albumin from the vascular to the extravascular space. By contrast, in Protocol B only blood-free tissue is analysed. Changes in $\text{PI}_B$ therefore represent a change either in permeability or in the size of the perfused capillary bed. In skeletal muscle following FR, there was no change in the CD of LPS-compared with sham-treated animals, implying that a rise in $\text{PI}_B$ was due to increased endothelial permeability. Other studies have corrected permeability indices for a potential decrease in the intravascular plasma volume induced by surgery or endotoxaemia [7,11]. If such decreases are valid (which is a questionable assumption not adopted in the present study, in view of potentially non-linear changes in plasma volume during sepsis), then we would have underestimated $\text{PI}_B$ in LPS-treated animals, and any correction factor would have enhanced the differences between LPS- and sham-treated animals. Thus the two protocols provide important complementary information regarding blood–tissue transport of albumin, in terms of endothelial permeability and alterations in compartmental distribution volumes.

In non-FR groups, $\text{PI}_A$ values in control lung, heart, liver and kidney showed close agreement with previously reported values for non-septic rodents [12]. In the present study higher values in skin (14.5 compared with 2.0) and skeletal muscle (7.0 compared with 2.0) may be due to indirect effects of higher doses of sodium pentobarbitone (60 mg/kg compared with 35 mg/kg, intraperitoneal) on the circulation, or the possibility of a low-grade systemic inflammatory response due to surgical cannulation and mechanical ventilation.

Counter-intuitively, LPS reduced $\text{PI}_A$ in most tissues. A possible explanation is that $^{131}$I-albumin was not retained intravascularly due to increased permeability, and tissue uptake of $^{131}$I therefore rose, producing a fall in the 60 min/5 min uptake ratio. This was not apparent in all organs. Increases in $\text{PI}_B$ after endotoxaemia were tissue-dependent; a finding consistent with previous studies employing different protocols, such as a single plasma volume marker (labelled albumin) and chromium-labelled red blood cells, to estimate intravascular volume. While this precludes quantitative comparisons, the pattern of variations was similar. Thus skin, skeletal muscle, kidney (medulla and cortex), mesentery and lung showed the most marked and significant increases in $\text{PI}_B$ following LPS in the present study. Small intestine, skeletal muscle, skin, heart and lungs (in decreasing order) displayed the highest percentage tissue/blood albumin distribution volume ratio following LPS infusion in a previous study [8], while the stomach, kidneys, lungs, skeletal muscle and large intestine (in decreasing order) showed the highest albumin leakage index (i.e. ratio of extravascular $^{1}$-labelled albumin to intravascular red blood cell volume) in another [6]. A greater $\text{PI}_B$ in mesentery from LPS-treated animals might have been anticipated, as has been reported in other species [13,14]. Significant protein loss into the peritoneal fluid and intestinal lumen may have reduced the overall retention of albumin within the mesenteric interstitium [8], thereby causing $\text{PI}_B$ to underestimate the true permeability of the mesenteric vascular bed for albumin.

FR increased the individual tissue distribution volume for each tracer, as described previously [15,16], although $\text{PI}_B$ did not rise significantly. Further, FR increased the number of tissues displaying LPS-induced rises in $\text{PI}_B$ and showed a trend towards reducing the metabolic base deficit, particularly after LPS. Changes in microvascular recruitment do not explain these changes. Thus FR may theoretically improve the maldistribution of microcirculatory flow associated with reduced CD in sepsis [17], in spite of normal global skeletal muscle perfusion in endotoxaemia [3]. However, data from the present study suggest that, while FR improves CD in non-septic animals, the increased heterogeneity of perfusion in sepsis is unaffected by FR. Mechanisms other than hypovolaemia, for instance microvascular thrombosis, must therefore be primarily responsible for capillary shutdown and redistribution during sepsis. Saline infusion showed a trend to improving endotoxin- and 1-NAME-induced base deficits, results that complement the beneficial effects of FR demonstrated in other studies [5,18,19]. These findings serve to reiterate the clinical importance of adequate FR in sepsis.

The HI did not change after intervention. FR may not have overcome insensible losses due to endotoxaemia, although in previous studies the fluid loading regime produced favourable alterations in microcirculatory tissue oxygenation, despite not increasing the cardiac output [3]. Alternatively, the tissue samples may not have shown large enough changes in tissue hydration to be identified. Indeed, tissue oedema was not evident following endotoxin or FR. It may not have been manifest during the experimental time frame, or endotoxaemia may have increased clearance of 1-albumin and water from the interstitial space [8]. Alternatively, interstitial fluid may have moved back into the vascular space across freely permeable capillaries [20,21].
1-NAME, a non-selective NOS inhibitor, had a pressor effect, increasing MAP when administered as an intravenous bolus. This effect was most evident after LPS, but was not sustained, possibly due to increasing production of iNOS-derived NO and its progressively increasing vasodilatory effect. 1-NAME attenuated the LPS-induced rise in $\Pi_{H}$ in the kidney and mesentery. Further, 1-NAME reduced the LPS-induced increase in lung $\Pi_{H}$ significantly, consistent with the effects of selective NOS inhibition in a similar model using Evans Blue-labelled BSA as a marker of permeability [22], while increasing it in the respective control group. 1-NAME had apparently detrimental systemic effects; the metabolic base deficit tended to worsen. This might reflect an 1-NAME-induced decrease in cardiac output, or even diminished microvascular blood flow [23]. These results suggest that iNOS-derived NO modulates increases in microvascular permeability to albumin in certain tissues, particularly the lung, in sepsis. The data are broadly supported by other studies demonstrating decreased permeability to protein in a range of tissues, albeit following selective iNOS inhibition [22,24–26]. By contrast, administration of $N^\text{G}$-monomethyl-L-arginine prior to, or 1-NAME concurrently with, LPS increased the intestinal permeability to albumin in rats [27,28]. The explanation for these apparent discrepancies is probably the different timing of interventions. Indeed, the timing of NOS inhibition in relation to the septic insult appears critical to its effect, such that the NOS inhibitor, irrespective of selectivity for iNOS, may reduce the sepsis-induced plasma leakage if used at an appropriate time after the induction of sepsis. Thus 1-NAME augments early endotoxin-induced gastrointestinal microvascular injury, but reduces the later injury associated with increased iNOS activity [28,29]. However, non-selective NOS inhibitors may have potentially deleterious consequences for tissue oxygenation in regional microvascular beds.

In summary, experimental sepsis induced by endotoxin had a significant tissue-dependent effect on transvascular albumin exchange. FR increased the range of tissues with increased albumin transport and tended to reduce the metabolic base deficit, although not apparently by microvascular recruitment. Low-dose, non-selective NOS inhibition reduced sepsis-induced albumin uptake within the lung parenchyma, but worsened the metabolic base deficit. These data implicate iNOS-derived NO as a cause of increased vascular permeability to protein in sepsis. Moreover, they suggest differential actions of NO between the lung and systemic tissues in this respect.

ACKNOWLEDGMENTS

S.S. and P.B.A. were supported by the British Heart Foundation.

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Received 28 June 2000; accepted 26 September 2000

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